Example 1, which teaches a method for screening for compounds that induce or inhibit nuclear translocation of a DNA transcription factor (See Column 23, lines 5-21).

Example teaches a method for determining the distribution of a transcription factor by a different method than the instant claimed method. The Dunlay patent teaches that after auto-focusing, an image of the nuclei is acquired and used to create a mask of the cell using any of a variety of known thresholding methods (See column 15, lines 48-67 and column 16, lines 1-10). The mask is labeled with a blob-labeling algorithm, so that each object (or blob) has a unique number assigned to it. Morphological features, such as area and shape, of the blobs are used to differentiate blobs likely to be cells from those that are considered artifacts. The user then pre-sets the morphological selection criteria by either typing in known cell morphological features or by using the interactive training utility. If objects of interest are found in the field, images are acquired for all other active channels, otherwise the stage is advanced to the next field in the current well. Each object of interest is located in the image for further analysis. Certain software is used to determine if the object meets the criteria for a valid cell nucleus (or other sub-cellular compartment) by measuring its morphological features (size and shape). For each valid cell, the XYZ stage location is recorded, a small image of the cell is stored and features are measured (See column 16, lines 14-30).

In accordance with the Dunlay et al. process, valid nuclear masks are identified and each valid nuclear mask is eroded to define a slightly smaller nuclear region, which is then used to extract the transcription factor's distribution in the nucleus. The nuclear mask is then dilated in two steps to define a ring shape region around the nucleus, which represents a cytoplasmic area. The average antibody fluorescence in each of these two regions is determined and the difference between the two (NucCyt difference) is noted. A change in the NucCyt difference in the presence of a test compound compared to in the absence of the compound provides an indication that the compound is an agonist or antagonist of the transcription factor.

Example 9 describes additional translocation assays. For example, the use of fluorescence anisotropy imaging microscopy (Gough and Taylor (1993), *J. Cell Biol.*

121:1095-1107) is suggested for measuring test-compound dependent movement of the fluorescent derivative of profilin between the cytoplasm and membrane (See Column 3, lines 64-67 and Column 34, lines 1-12). In addition, certain masking strategies are suggested for determining Rho-RhoGDI complex translocation to the membrane (See Col.umn 34, lines 13-34); ß-arrestin translocation to the plasma membrane upon G-protein receptor activation (See Column 34, lines 35-67) and VSVG protein translocation from the endoplasmic reticulum to the Golgi (See Column 35, lines 13-56). The integrated brightness per unit area under each mask is used to form a translocation quotient and a change in the quotient in the presence of a test compound relative to in the absence of the test compound indicates that the test compound is an agonist or antagonist of translocation for the particular molecule.

The Dunlay et al. patents, however, do not teach or suggest the instant claimed methods in which the intensity of particular stains is analyzed to actually identify sub-cellular compartments, such as the cell nucleus or cytoplasm or even virtual compartments. In other words, in the instant claimed process, the compartment itself is defined by molecular interactions.

In contrast, the Dunlay et al. process, employs software that allows the user to pre-set morphological selection criteria (e.g. the nucleus, cytoplasm or cell membrane) by either typing in known cell morphological features (e.g. area or shape) or by using the interactive training utility.

In addition, the Dunlay et al., patents do not teach or suggest the instant claimed process for determining if a biomarker is present within a particular sub-cellular compartment by subtracting a percentage of the intensity value for each pixel location in the second image from the intensity value of the same pixel location in the first image to obtain an adjusted intensity value, indicative of the biomarker within the subcellular compartment.

Rather, as described above, the Dunlay patents teach methods that rely on obtaining average measurements of a particular labeled biomarker in two different subcellular compartments and noting the differential intensities of the labeled biomarker

in the two locations (e.g the NucCyt difference), determining the brightness per unit area under each subcellular mask to obtain a translocation quotient or using fluorescence anisotropy imaging microscopy (Gough and Taylor (1993), *J. Cell Biol.* 121:1095-1107) to measure test-compound dependent movement of the fluorescent derivative of profilin between the cytoplasm and membrane.

Since the Dunlay et al., patents do not teach each and every element of the instant claims, the patents do not anticipate the claims and the rejection of claims under 35 U.S.C. § 102(e) should be withdrawn.

Rejection of claims 1-32 and 39 under 35 U.S.C. §103(a) as being unpatentable ovre Dunlay et al. (U.S. Patent No. 6,727,071)

The Examiner has rejected claims 1-32 and 39 under 35 U.S.C. §103 (a) as being unpatentable over Dunlay et al., as applied above in the rejection under 35 U.S.C. § 102(e). In particular, the Examiner acknowledges that Dunlay et al. does not specifically provide an example of labeling cell compartments with additional stains (i.e. third and fourth), but states that these embodiments are obvious in view of the Dunlay disclosure pertaining to methods for labeling cellular compartments (Col. 39-43).

In addition to the differences noted by the Examiner, as pointed out above, the Dunlay et al. patents do not teach of suggest the instant claimed methods of: (i) identifying subcellular compartments by measuring the intensity of particular stains in various locations within a cell, or (ii) determining the presence of a biomarker within a particular sub-cellular compartment by subtracting a percentage of the intensity value for each pixel location in the second image from the intensity value of the same pixel location in the first image to obtain an adjusted intensity value, indicative of the biomarker within the sub-cellular compartment.

The instant claimed method

Because one of skill in the art would not arrive at the instant claimed methods based on the teachings of the Dunlay et al., patents, Applicants respectfully submit that the claimed invention cannot be deemed obvious in light of the teachings of U.S. Patent

No. 6,727,071 and that therefore, the rejection of claims 1-32 under 35 U.S.C. §103 (a) should be withdrawn.

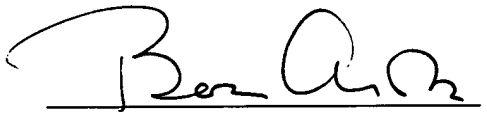
CONCLUSION

For the foregoing reasons, Applicants respectfully request reconsideration and withdrawal of the pending rejections. Applicants believe that the claims now pending are in condition for allowance, and notification of such is respectfully requested.

The Commissioner is hereby authorized to credit any overpayment or charge any deficiencies to Deposit Account Number **06-1448, Reference YUA-001.01**.

If, for any reason, a telephonic conference with the Applicants would be helpful in expediting prosecution of the instant application, the Examiner is invited to call Applicants' Agent at the telephone number provided below.

Respectfully submitted,
FOLEY HOAG LLP



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